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Use of Flumequine against Furunculosis in Salmonids:
Therapeutic Trials and Practical Perspectives

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Aeromonas salmonicida-induced furunculosis is currently the primary cause of bacterial death in freshwater-reared salmonids. Treatment is at present the only available means of control with the oral route as the only practical mode of administering drugs to specimens maintained in large amounts of water. The range of antibiotics that may be used is limited to the tetracyclines, chloramphenicol, sulfanilamide compounds (occasionally combined with trimethoprim) and some furan substances. Since there is a fairly substantial failure rate due to drug resistance, any new molecule that can be assimilated by ingestion and is capable of impeding the development of Aeromonas salmonicida may be considered a valuable means of combating the disease, provided, of course, that active doses are compatible with a reasonable cost price.

I. Characteristics of Flumequine

Flumequine is a quinoline compound (Fig. 1) and was synthesized by Riker Laboratories. It is widely used as an antibacterial agent in humans (marketed under the name of Apurone) and in various animal species (Benothmane, 1979). It is bactericidal, acting specifically against Gram-

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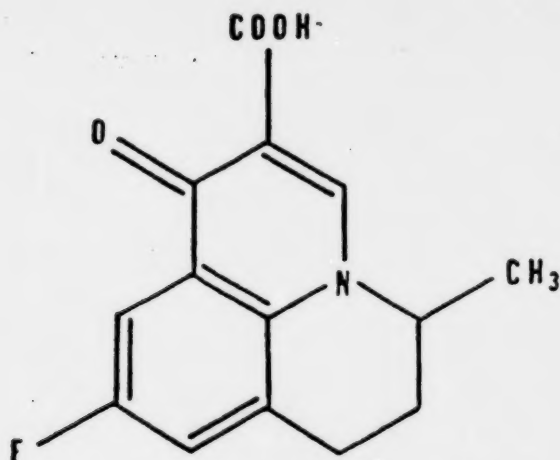


Fig. 1 The Flumequine Molecule

negative and staphylococcal organisms. Colibacilli are particularly susceptible to the substance which is also active against the following genera: Aeromonas, Enterobacter, Kebsiella and Yersinia (J. Oudar, Expert's clinical report). Because of its small molecular size, it passes readily through the intestinal barrier following oral doses and is very quickly metabolized. Experiments in higher animals have shown that it is rapidly eliminated (in less than 48 hours) and that the chances of resistance are very remote. To date, there has been no evidence of extra-chromosomal resistance. There is virtually no toxicity since whatever signs have been recorded have occurred with doses several hundred times above the recommended dose.

Over the past few months, flumequine has been systematically included in the sensitivity studies carried out in the laboratory. With every identified Aeromonas salmonicida strain, the substance has produced a growth inhibition disc with a diameter of over 20 mm. As these findings indicate susceptibility to flumequine, we designed a series of therapeutic trials in a laboratory setting. A relatively well standardized infection model

developed by Michel in 1980 enabled us to undertake this preliminary step which is essential to any study in the field.

II. Therapeutic Trials in Trout: Procedure

The Subjects

Rainbow (Salmo gairdneri) and fario (Salmo trutta) trout fingerlings were selected. There was some variation in mean weight over time, i.e., 7 to 13 g for the rainbow trout specimens and 5 to 6.5 g for the fario trout. The LD50 values for bacterial strains that were determined at the onset of the study no longer applied at the end and it was consequently necessary to rectify dosage. A few days before the study, the animals were divided into groups of 30 specimens in 12-litre flow-through tanks in which the feed water was heated to 15°C. The rate of flow was regulated to roughly 30 litres/hour.

The Bacterial Strains

Two pathogenic strains of A. salmonicida, which had been isolated and were maintained at Grignon, were used for the study. Their virulence expressed in terms of LD50 values was determined by the method of moving averages (Meynell and Meynell, 1970) before the study was undertaken and yielded the following results:

TG 36/75: rainbow trout 700 b (IM)

TG 72/78: rainbow trout 800 b (IM)

fario trout 400 b (IM)

Virulent suspensions were obtained after roughly 18 hours' growth on a

trypticase soya broth that was stirred at 22°C. Doses were adjusted by dilution in physiological water after the turbidity was read on the spectrophotometer (525 nm).

Experimental Infection

With the model we used, an intramuscular injection of a known quantity of bacteria produces an acute infection within 10 days in water maintained at 15°C. A 0.1 ml solution was injected under anesthesia with 100 ppm MS 222 (Sandoz).

Two infective doses were selected in relation to the LD50. The first corresponded to the LD50 proper and the second to a dose ten times as high, which is an unlikely situation under normal conditions.

Monitoring Flumequine Tolerance in Fingerlings

In order to determine whether there was any risk of toxic reactions to Flumix, doses ten times the recommended dose for mammals were administered per os to rainbow trout fingerlings for 15 days prior to the study proper. Single doses of up to 2 g pure flumequine per kilo of live weight were also given to lots of 10 fingerlings.

Treatment Procedure

Flumix (Flumequine in powder form, designed for oral use) was mixed with the standard food pellets. Treatment lasted for 6 days. During the course of the study, the reference dose D was based on the mammalian dose, i.e., 20 g Flumix (3% flumequine) per 100 kg live weight/day. Since the

animals were given the equivalent of 1% live weight in food, dose D was equal to 20 g/kg food. We also administered doses that were twice ($D \times 2$) and four times ($D \times 4$) as high. It is standard practice for antibiotics to be administered to fish in double the doses used in mammals.

Food was distributed twice daily and treatment usually started on the day the study began. In one experiment, however, treatment did not begin until 20 hours after induction of the infection.

III. Results

Absence of Acute Toxicity

No abnormal findings were recorded in the behaviour of animals given 2 g Flumix/kilo of body weight per day for 15 days nor in specimens treated with single doses of 0.5 to 2 g pure flumequine. This led us to conclude that flumequine was devoid of acute toxicity in trout.

Therapeutic Effectiveness of Flumequine

Specimens Treated from the Onset of the Study

In every instance, flumequine significantly lowered the mortality rate recorded in control animals. The results are given in the following tables in terms of different parameters. The degree of protection is expressed as the difference D in furunculosis-induced losses (in %) between control and treated animals.

a) The Species (Table 1)

With daily doses of 40 g/100 kg live weight, i.e., the usual dose in fish, fario and rainbow trout fingerlings were equally well protected, the protection rate being roughly 60% for both species.

Table 1

Prevention of Furunculosis in Two Trout Species (*A. salmonicida* strain 72/78. Infection dose $\approx$ 1 LD50)

Species	Rainbow Trout		Fario Trout	
Infection dose	940 b IM		430 b IM	
Dose of flumequine	Controls D x 2		Controls D x 2	
Mortality rate	22/30	2/30	18/30	0/29
%	73%	6.5%	60%	0%
Δ	66.5%		60%	

Δ = (% controls - % treated animals)

b) The Bacterial Strains (Table 2)

Table 2

Protection against Two Different *A. salmonicida* Strains in Rainbow Trout (lots of 30 fingerlings)

Strain source	TC 36/75						TC 72/78					
	720 b IM (= 1 D _L 50)			720 b IM (= 10 D _L 50)			940 b IM (= 1 D _L 50)			940 b IM (= 10 D _L 50)		
Dose of flumequine	1	D x 2	D x 4	1	D x 4		1	D x 2	D x 4	1	D x 4	
Mortality rate	15	0	0	23	3		22	2	1	28	1	
Survival rate	50%	0	0	76.5%	10%		72%	65%	3%	80%	3%	
Δ		50%	50%	66.5%			66.5%	76%		80%		

In a study conducted exclusively in rainbow trout fingerlings using different bacterial concentrations as well as a range of flumequine doses, it was found that the compound afforded slightly better protection against strain TG 72/78 than against strain TG 36/75. This can hardly be accounted for by differences in virulence which is only very slightly higher in the latter strain. Experimental conditions may have been a factor since the LD50 of TG 72/78 killed close to 75% of the controls (of Infection Doses). It was clear that flumequine was effective against both strains.

c) Therapeutic Doses (Table 3)

Table 3

Effect of the Therapeutic Dose in Protecting against Furunculosis (Infection dose $\approx$ 1 LD50)

Strain Souche	TG 36/75			TG 72/78					
Espèce Species	Arc-en-Ciel						Fario		
Dose de germe infection dose	720 b 1M			940 b 1M			430 b 1M		
Dose de flumequine dose of flumequine	1	D = 2	D = 4	1	D = 2	D = 4	1	D	D = 2
Moribonds moribonds pourcentage	15/30	0/30	0/30	22/30	7/30	1/30	18/30	1/28	0/28
	50%	0	0	73%	6.5%	3%	60%	3.5%	0
Δ		50%	50%		66.5%	70%		50.5%	60%

Irrespective of the dose of Flumix administered, no significant differences were recorded in the same species infected with the same strain. Even mammalian doses (D) seemed highly effective. In practice, however, we deem it advisable to use doses of 40 g/100 kg live weight to compensate for possible errors in assessing proper dosage and for the unpredictable feeding behaviour of the fingerlings.

d) Infections Doses (Table 4)

Table 4

Effect of the Infection Dose on the Protection Rate (Rainbow trout fingerlings, lots of 30 fish)

Strain	Souche	TG 3: 75 F				TG 72/78 R			
		720 b IM (= 1 DL 50)		7200 b IM (= 10 DL 50)		940 b IM (= 1 DL 50)		9400 b IM (= 10 DL 50)	
Dose d'épreuve infection dose		T	D x 4	T	D x 4	T	D x 4	T	D x 4
Dose de fluméquine dose of flumequine									
Mortalités mortality rate		15	0	23	3	22	1	28	1
Pourcentages %		50%	0%	76.5%	10%	73%	3%	93%	3%
Δ		50%		66.5%		70%		90%	

Since the rate of protection was proportional to the size of the infection dose, the unprotected (control) animals suffered higher losses. Protection was highly effective even in the case of infection capable of destroying virtually the entire stock.

The Importance of Properly Selecting Treatment Time in Relation to the Development of Infection

Table 5

Comparison of Results of Treatment Given at the Time of Infection and Treatment Instituted 20 hours later (Strain TG 72/78; infection dose $\approx$ 1 LD50; t_0 = infection time)

Species Espèce	Rainbow trout Truite Arc-en-Ciel				Fario trout Truite Fario					
Début du traitement (en heures) onset of treatment (hrs)	L		L + 20 h		L			L + 20 h		
Dose d'épreuve infection dose	940 b IM (1)		2500 b IM (1)		430 b IM			860 b IM		
Dose de fluméquine dose of flumequine	T	D x 2	T	D x 2	T	D	C x 2	T	D	D x 2
Mortalité mortality rate	22/30	2/30	22/30	14/30	18/30	1/28	0/29	24/29	21/28	2/29
Pourcentages %	73 %	6.5 %	73 %	46.5 %	60 %	3.5 %	0	83 %	75 %	7 %
Δ	66.5 %		26.5 %		56.5 %			60 %	8 %	

(1) The difference in the LD50 was due to an increase in fingerling weight during the course of the study

Results showed that the drug lost some of its effectiveness when administered close to one day after the onset of infection as compared to treatment instituted at the time of infection. The one exception in the last column of Table 5 (dose D x 2 in fario fingerlings) indicates that protection was effective in the aquarium involved. This exception, which was attributed to tank conditions, demonstrates that the effectiveness of flumequine cannot be questioned. Since sick fish are known to stop feeding, the reduced effectiveness of treatment instituted 20 hours after the induction of infection was probably due to the fact that individuals with more advanced infection refused to eat. Like all anti-infection agents administered orally to fish, flumequine acts only in animals capable of taking nourishment.

IV. Possible Practical Uses in Fish Breeding Operations

Results show that flumequine may be a highly useful means of combating furunculosis provided that the general rules for any antimicrobial agent administered in fish-rearing operations are obeyed, i.e., early administration of adequate doses given over prolonged periods of time (Michel, 1978).

In fish, we recommend the powder form (Flumequine Powder or Flumix) which contains 3% of the active principle, flumequine. It may be obtained from veterinarians, if necessary (500 g, 1 or 5 kg jars) and prepared in proper concentrations, as described above. Since the fish are accustomed to a certain type of food in a certain pellet size, for reasons of palatability, it is preferable to administer the substance with the food.

Flumix may be mixed with the food pellets (recommended dose = 40 g/kg food) in a washing machine barrel or cement mixer until the mixture is perfectly homogeneous. Sunflower seed oil is then added in doses of 1 litre/50 kg food. The mixture is not used until the pellets have become uniform in colour. Since flumequine is highly stable, the amount of drug-treated food needed for 6 days of treatment may be prepared on the very first day. As with any other foodstuff, the preparation should be kept in a cool, dry place.

The fish should be fed in amounts equal to 1% of their live weight, divided into two (morning and evening) meals. This procedure prevents wastage and provides high and fairly constant tissue concentrations of the active ingredient throughout treatment.

Conclusions

Flumequine proved active during our experimental trials, yet no untoward effects were detected even with doses five times as great as the doses we recommend. If used correctly, this formula should prove a valuable means of combating bacterial diseases occurring in fish-rearing operations. The drug is perfectly accepted by the animals and is unquestionably effective against the furunculosis-inducing agent. Furthermore, it is not any more costly than most the usually advocated treatments.

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